Notes

Culture Maintenance of Vanessa atalanta rubria (Nymphalidae)

Many lepidopterists do not have access to the laboratory facilities frequently referred to in publications dealing with butterfly culture. While sterile, controlled laboratory conditions may certainly be preferred for long-term maintenance of healthy cultures, they are not a necessity for all species. There is a great deal of work which can be achieved by those with only residential, indoor facilities as places of work.

The project reported in this article was performed in a one-bedroom upstairs apartment which received only 90 to 120 minutes of afternoon sunlight each day. I wished to determine the conditions necessary to establish and maintain a culture of *Vanessa atalanta rubria* (Fruhstorfer) indoors. Because of airborne particles from cooking, cleaning, and other domestic activities, plus fluctuations of temperature, light, and humidity, this environment was neither sterile nor controlled. However, from February to July 1982 I was able to raise four continuous generations of butterflies. The following procedures I hope will motivate and assist others to experiment with the same or other species.

If rearing is to be done in the same room a collection is kept, it is essential that all VaponaTM pest strips, if they are being used, be removed from the residence. This insecticide is very effective even when used in "airtight" cabinets: the vapor escapes when these are opened. In the genus *Vanessa* when early stages are exposed, ova do not hatch and larvae gradually lose the ability to hold onto their foodplants and react by "spinning webs in the air" before dying. The residence must be aired well before livestock is brought inside. I have used paradichlorobenzene (PDB) in small quantities in airtight cabinets to protect my collection without harm to the cultures in the same room. If PDB odor was present after opening the cabinets, the room was quickly aired.

In nature, Vanessa butterflies hilltop and sun themselves in the afternoon for territorial reasons noted by Bitzer and Shaw (1979(80), Territorial Behavior of the Red Admiral, Vanessa atalanta (L.) (Lepidoptera: Nymphalidae). J. Res. Lepid. 18:36-49) and in preparation for courtship and mating according to Shields (1967, Hilltopping. J. Res. Lepid. 6:69-178). Therefore, a flight cage should be placed in a window with afternoon sunshine. The flight cage used was modified from that used by White (1981, pers. comm.) and measures $51 \times 51 \times 122 \text{ cm}$ (20 $\times 20 \times 48$ inches), constructed of one-inch wood frames and nylon netting on all sides except the bottom which is hardboard. An inexpensive cage can be made from a large cardboard box by cutting out the side and top panels, leaving a one to two-inch margin for strength on each edge to which the netting is glued.

Captive Vanessa atalanta feed throughout the day. A honey-water mixture was provided in the proportion of 6 to 9 ml of honey to 190 ml of water (1 to $1\frac{1}{2}$ teaspoons honey in 1 cup of water) and was made daily. The mixture was poured into a petri dish and white, unscented tissue paper was added until most of the liquid was absorbed and the tissue remained saturated.

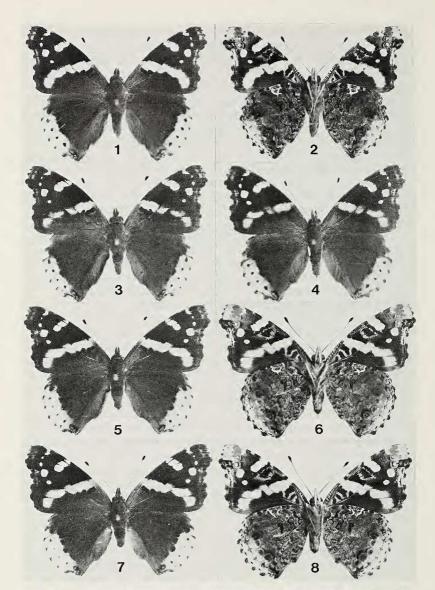
Freshly cut (with a sharp razor blade) stalks of the foodplant *Urtica holosericea* Nuttall (Stinging Nettle) were placed in small jars of water and placed into the cage every one to three days. On 19 February 1982 ca. 20 larvae of V. atalanta rubria were collected on U. holosericea growing along a seasonal foothill creek in Barlow Canyon, Ventura Co., California. These were reared to adults which were then placed into the flight cage with fresh nettles and honey-water. The first matings occurred four days after emergence and took place at or soon after sunset. "Sunset" in the room was 90 to 120 minutes before the actual sunset because the neighboring building blocked out the late afternoon sun. The male butterfly located a female, approached her along one side, and vibrated his wings in a nearly closed position. He held one of his antennae forward with those of the female and the other directed posteriorly to her abdomen. He then curled his abdomen so that the posterior tip was directed forward with the genitalia flared. He then attempted copulation. Even if the female walked away from the male, he would run after her in this position. If copulation was successful, the pair became quiescent for the duration of the mating process. Most often this lasted 90 minutes, but occasionally longer, even overnight matings occurred.

V. a. rubria prefers mating at or after sunset, and this twilight effect can be lengthened in duration by using a floodlight above the cage. Both GE 75-watt Reflector Spot and GE Plant Gro and Sho 75-watt Spot lights were used successfully, one at a time. The light was mounted 15 cm above one side of the cage and directed slightly downward across the top netting to the opposite corner. This gave the butterflies a longer period each day to court and mate.

Even within the confines of the flight cage, the males displayed territorial chase behavior, although the flights were limited in area. Very often the males would run after each other instead of fly, and this behavior was quite amusing to observe because it was so unexpected. The first male sat head down, wings closed, on the sunlit side of the cage, and would "watch" another male flying around the cage interior. I use the term "watch" because this male would follow the flight of the other either by short, lateral head movements or by turning his body to direct his eyes forward. If the second male landed nearby (within ca. 30 cm), the first male would run over to him and tap his mesothoracic legs upon the thorax or wing bases of the second male until the second male either ran or gave chase. During times when no males were flying in the cage, a "restless" male seeking a chase would run to another and initiate a chase in the same manner. Less often, three or four individuals would be involved in the same chase. When behaviors such as these are observed in a cage, it is an indication that the butterflies are quite content and courting and mating will most likely take place.

The netting on the side of the cage facing the window was the primary locality for the courting and mating activities, so the foodplants were set back to the middle of the cage. The honey-water dish was placed on the cage floor. Elevated perching sites such as tall, narrow boxes wrapped in paper towels were readily used by sunning individuals, and when honey-water was sprinkled on these the butterflies fed eagerly.

The minimum number of adults in a cage where mating took place was one male and two females. The maximum number was not determined, but cages with 15 to 20 butterflies produced good results. With the use of a floodlight, a total of 8 successful matings occurred among a total of 16 individuals, with some males mating twice. Indoor temperatures during courtships and matings varied from 24° to 27°C. Hand pairing was not attempted.



Figs. 1-8. Vanessa atalanta rubria, all reared. Figs. 1 & 2: typical, normal male from Calif.: Ventura Co.: Barlow Cyn. Elev. 500 ft., 17 December 1978. Figs. 3-8: variant phenotypes from culture. Filial generation, sex, dates of emergence (all 1982), and brood labels: Fig. 3, F₃male, 9 July, 3B; Fig. 4, F₃ female, 2 July, 3BDF; Fig. 5, F₂ female, 28 May, 2DC2; Fig. 6, F₂ female, 28 May, 2DC2 (different specimen than Fig. 5); Figs. 7 & 8, F₃ female, 5 July, 3B.

Wild collected males of both V. a. rubria and V. annabella (Field) did not adjust to the confinement of the cage. They showed no interest in the unmated females and quickly destroyed their wings flying against the netting. Among the reared butterflies, mating began one day after emergence, but could take place for the first time a week after emergence.

Females began to lay eggs one day after mating. This species is very prolific and can lay over 300 eggs. The duration of the egg stage is four days at indoor temperatures of 24° to 27° C, so on the third day leaves with ova were placed into rearing containers. The foodplant leaves can be cut up when specific numbers of ova are desired in each container. As some of the females aged and became familiar with the cage interior, they gave up any finesse they had with the oviposition process and simply fluttered to the general vicinity of the foodplants, dropped into them, and began laying eggs wherever the abdomen contacted a plant surface. Many laid eggs on the netting, and the resulting larvae were transferred to containers with the use of a fine brush.

Larvae were reared in plastic boxes measuring 111 X 111 X 39 mm (BioQuip Cat. No. 1182A). Six to eight sheets of white, unscented bathroom tissue were placed in the bottom and slightly dampened, and fresh foodplant leaves were placed inside. Setting one flat leaf on top of another provides an easy situation for nest construction by hatching larvae. The tissue was changed and the old foodplant replaced with new as often as needed. The higher humidity inside these boxes keep the foodplants fresh for several days, and larvae mature quickly. Unfortunately these same conditions promoted the development and spread of disease, and great care was taken to remove dead or dying larvae. What was believed to be a polyhedral virus (White, 1981, and Taylor, 1982, pers. comms.) caused heavy mortality in the last instars and pupae in all the generations reared in this project. Had sterilization techniques been used on the ova and foodplants, it is possible the disease could have been stopped. Sterilization procedures currently in use for Vanessa cardui (Linnaeus) cultures by White (1981, pers. comm.) require washing the ova in a 5-10% Chlorox solution with 2 drops of jet Dry detergent per 16 oz. of Chlorox solution for 10 minutes, draining, and washing in water for 10 minutes. This procedure also removes the ova from the leaf surfaces, so the ova must be collected with filters. Foodplants must similarly be surface decontaminated.

If sterilization methods are not used, the disease can be largely avoided by transferring the fourth instar larvae from the rearing boxes to cut stalks of foodplants in water in large, airy rearing cages. Contrary to Stone and Midwinter (1975, Butterfly Culture. p. 27, Blanford Press, Dorset, Great Britain, culture instructions for *Aglais urticae* (L.) referred to from *V. atalanta*, p. 31), larvae did not die when reared on cut nettles placed in water. Younger larvae can be transferred if the disease should begin to appear earlier. Rearing cages are preferred if large numbers of larvae are to be reared simply because they are easier to clean than the great number of plastic containers needed to house an equal number of larvae.

The plastic rearing boxes are deep enough to allow the mature larvae to hang freely for pupation from the lid. The larvae usually secure all nearby surfaces with silk webbing before hanging, and this can be carefully cut permitting removal of the lid. Following hardening of the pupa (one day), an area surrounding the cremaster and its silk foundation is scored with a sharp object. The resulting 1-2 cm circle of silk with the attached pupa is removed by pressing adhesive tape onto the silk foundation alongside the cremaster and pulling the tape off again. The entire foundation usually sticks to the tape which is then reapplied onto the lower surface of a larger cardboard platform which in turn is placed into an emergence cage. Eclosing adults are then examined on a daily basis and those with characteristics desired for breeding are selected. Fresh adults are easily examined when a pencil or probe wrapped in a paper towel dipped in honey-water is placed at the front of the butterfly's mesothoracic legs. It begins feeding and displaying its wings after its feet touch the food.

This culture was terminated in the third week of July 1982 at the end of the third generation as the foodplant resources were in seasonal decline. Balcom Canyon, near Santa Paula, Ventura Co., California, is the site of very large colonies of *Urtica holosericea* and was the source of all foodplants used in this project.

While the main purpose of this work was to establish conditions necessary for maintaining a culture of V. a. rubria, some mass selection was accomplished. In one culture, adults with the most constricted red band on the forewing were selected for mating, and four separate lines were maintained. The most extreme results are shown in Figs. 3 and 4. In a second line of breeding, two pairs of Fo adults with a scale deficiency were bred together, but all the ova failed to hatch. In a third line, an interesting variation occurred in the second generation which differed from typical V. a. rubria in the following ways (Figs. 5 and 6): a slightly modified wing shape; on the upperside, a wider marginal red band on the hindwing with protruding ocelli; and on the underside, increased brown coloration of most of the mottling pattern elements and reduced size of the ocelli. This variant was informally referred to as "brunnea" in the culture. Two pairs of adults of this variation were bred together and from the two females over 800 ova were obtained in two weeks. Unfortunately, exact numbers of the resulting phenotypes were not recorded as the entire project had reached such overwhelming numbers by this time that except for simple cage maintenance, all other activities were severely limited. General observations of the F₂ showed the majority to be phenotypically like the F₂ parents, but many had greater purplish-blue development in place of the brown, and others had the hindwing upperside band yellowish instead of red (Figs. 7 and 8). Another variant unlike the parents occurred in about one-fourth of the F_2 : it had a bright pinkish-orange cast to the upperside bands, a nearly normal underside, and normally-shaped wings.

The few examples above only hint at the multitude of possibilities for genetic research, in addition to revealing hidden genetic variability. The use of a single gravid female to start a culture would greatly simplify interpretation of results, and the use of an artificial diet would permit the maintenance of the culture throughout the year.

I am sincerely grateful to to Carlos White of Insect Lore Products, Shafter, California, for his many helpful suggestions for establishing cultures of Vanessa butterflies. Sir Cyril Clarke, Merseyside, England, also generously shared techniques he developed for culturing Vanessa a. atalanta (L.) and V. annabella. Dr. Orley Taylor made helpful suggestions for developing an artificial diet and sterilizing ova.

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